products in the second and third vessels are mixed to bring together those products having random codon sequences at single codon positions. This mixing also reduces the product populations to three, which are the starting populations for the next round of synthesis. Similarly, for the third, fourth and each remaining position, each reaction product population for the preceding position are divided and a parent and random codon synthesized.

Following the above modification of codon-based synthesis, populations containing random codon changes at one, two, three and four positions as well as others can be conveniently separated out and used based on the need of the individual. Moreover, this synthesis scheme also allows enrichment of the populations for the randomized sequences over the parent sequence since the vessel containing only the parent sequence synthesis is similarly separated out from the random codon synthesis.

Other methods well known in the art for producing a large number of alterations in a known amino acid sequence or for generating a diverse population of variable or random sequences include, for example, degenerate or partially degenerate oligonucleotide synthesis. Codons specifying equal mixtures of all four nucleotide monomers, represented as NNN, results in degenerate synthesis. Whereas partially degenerate synthesis can be accomplished using, for example, the NNG/T codon described previously. Other method well know in the art can alternatively be used such as the use of statistically predetermined, or varigated, codon synthesis which is the subject matter of U.S. Patent Nos. 5,223,409 and 5,403,484.

Once the populations of altered variable region encoding nucleic acids have been constructed as described above, they can be expressed to generate a population of altered variable region polypeptides that can be screened for binding affinity. For example, the altered variable region encoding nucleic acids can be cloned into an appropriate vector for propagation, manipulation and expression. Such vectors are known or can be constructed by those skilled in the art and should contain all expression elements sufficient for the transcription, translation, regulation, and if desired, sorting and secretion of the altered variable region polypeptides. The vectors also can be for use in either procaryotic or eukaryotic host systems so long as the expression and regulatory elements are of compatible origin. The expression vectors can additionally included regulatory elements for inducible or cell type-specific expression. One skilled in the art will know which host systems are compatible with a particular vector and which regulatory or functional elements are sufficient to achieve expression of the polypeptides in soluble, secreted or cell surface forms.

Appropriate host cells, include for example, bacteria and corresponding bacteriophage expression systems, yeast, avian, insect and mammalian cells. Methods for recombinant expression, screening and purification of populations of altered variable regions or altered variable region polypeptides within such populations in various host systems are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992) and in Ansubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1998). The choice of a particular

vector and host system for expression and screening of altered variable regions will be known by those skilled in the art and will depend on the preference of the user. A specific example of the expression of recombinant altered variable region polypeptides is additionally described below in the Examples. Moreover, expression of diverse populations of hetereomeric receptors in either soluble or cell surface form using filamentous bacteriophage vector/host systems is well known in the art and is the subject matter of U.S. Patent No. 5,871,974.

The expressed population of altered variable region polypeptides can be screened for the identification of one or more altered variable region species exhibiting binding affinity substantially the same or greater than the donor CDR variable region. Screening can be accomplished using various methods well known in the art for determining the binding affinity of a polypeptide or compound. Additionaly, methods based on determining the relative affinity of binding molecules to their partner by comparing the amount of binding between the altered variable region polypeptides and the donor CDR variable region can similarly be used for the identification of species exhibiting binding affinity substantially the same or greater than the donor CDR variable region. All of such methods can be performed, for example, in solution or in solid phase. Moreover, various formats of binding assays are well known in the art and include, for example, immobilization to filters such as nylon or nitrocellulose; two-dimensional arrays, enzyme linked immunosorbant assay (ELISA), radioimmune assay (RIA), panning and plasmon resonance. Such methods can be found described in, for example, Sambrook et al., *supra*, and Ansubel et al.

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